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Effect of Cold Storage on the Survival, Sex Ratio and Longevity of Ectoparasitoid, *Bracon hebetor* (Say.) (Hymenoptera: Braconidae)

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ABSTRACT

The ectoparasitoid, *Bracon hebetor* (Say.) (Hymenoptera: Braconidae) is considered one of the most important biological control agents of several pyralid (Lepidoptera) pest insects. The effects of low temperature storage on the quality of adult parasitoids and their progeny were investigated by storing different life stages of the parasitoid (eggs, larvae, pupae and adult) at temperatures *i.e.*, 4±1°C and life sages of the wasp stored at 27±1°C were acted as control (12L:12D photoperiod) for 5, 10, 20 and 40 days. Our data reveals that number of surviving individual decreases as the storage period increases along with the rate of emergence which reduced from onward 10 days of storage and with decreasing temperature at 4°C. Highest survival of 24.00±1.41 was observed during storage of adult wasp at 4°C followed by pupae (20.00±1.41), however larvae and eggs could not survive to adult stage after 40 days cold storage treatment. Adult longevity decreased while sex ratio was female biased in all cases by increasing cold storage duration. Therefore, it may be concluded that adult stage of *B. hebetor* is more cold tolerant than all other stages and considered to be more suitable for short term storage.

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Authors' Contribution

ZUA conceived and supervised the research project. MA executed the experimental work. SKA statistically analyzed the data. FH contributed towards insect rearing and handling. AM established host parasitoid culture in the lab. MT drafted the manuscript.

Key words

Cold storage, Ectoparasitoid, Larval emergence.

INTRODUCTION

Bracon hebetor is a larval ectoparasitoid of many lepidopteran species, and a voracious feeder of stored grains and lepidopterous pest (Athanassiou and Eliopoulos, 2003; Darwish *et al*., 2003; Gupta and Sharma, 2004; Shojaei *et al*., 2006). The biology of *B. hebetor* has been intensively studied because of its suitability as a model organism: which is easy to rear in the laboratory and also has a great potential for being used as a biological control agent of many lepidopterous pests. It occurs naturally, throughout the world. Nikam and Pawar (1993) reported that *B. hebetor* can also be used as an important bio-control agent of *Helicoverpa armigera.* It is used in Turkmenistan, on cotton and to a lesser extent, in Uzbekistan, where they rely more on *Trichogramma pintoi* (Matthews, 1997). *B. hebetor*, was used to control the *Ecomyelios ceratoniae* on dates, in warehouses. The broad host range, high reproductive rate and short generation time makes *B. hebetor* an excellent candidate for biological control of many pyralid pests, including *G. mellonella.* The establishment of different storage methods of natural enemies is of great importance for the pliability and regulation of huge multiplication in the insectary (Greenberg *et al*., 1996; Leopold, 1998; Tezze and Botto, 2004).

Cold storage can permit a cost-effective production schedule, providing a means to conserve biological control agents when not immediately needed (Pitcher *et al*., 2002; Ayvaz *et al*., 2008). It also allows synchronized field releases of natural enemies during the critical stages of pest (McDonald and Kok, 1990; Venkatesan *et al*., 2000).

Many previous studies indicated that appropriate conditions to store parasitoids varies from species to species and should be studied in detail for each species separately prior to their use in biocontrol (Foerster *et al*., 2004; Tezze and Botto, 2004; Bayram *et al*., 2005; Kumar *et al*., 2005; Ayvaz *et al*., 2008). The practice of low temperature for the protection of stored products from insect contamination can be used as a substitute of chemical control (Fields, 1992; Johnson and Woffoard, 1991). The use of cold storage technique could be useful for industrial rearing of beneficial insects as well as for research under laboratory condition. So, identifying the best stage and storing duration of *B. hebetor* is essential for boosting its successful results as a commercial control agent for pyralid pests. The present research project was focused to regulate the suitable temperature and storage time and the life stage of parasitoid (*i.e.*, whether it is egg, larvae or pupae) *Bracon hebetor* (Say.) in cold conditions.

MATERIALS AND METHODS

Rearing and handling of experimental insects

Rearing and handling of experimental insects were carried out in Insect Molecular Biology Lab. Department

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of Entomology, UAF. The ectophagous larval parasitic wasp *B. hebetor* (Say.) (Hymenoptera: Braconidae) was reared in the laboratory on the late stage (5th instars) larvae of greater wax moth *G. mellonella* (Lepidoptera: Pyralidae) by following a slightly modified approach as described by (Anam *et al.*, 2015). Both the adults of the parasitoid, *B. hebetor* were collected directly from the berseem crop, *Trifolium alexandrium* L., along with the larvae, pupae and adults of the host, greater wax moth, *G. mellonella* from the infested bee hives located at the campuses of the University of Agriculture, Faisalabad, Pakistan. The collected parasitoids were identified on the basis of morphological characters by making comparison with the available literature. The host and parasitoid cultures were maintained in two separate glass jars, both placed at 27±1°C, 65±5% relative humidity (RH). *B. hebetor* was reared on 12/12 h light and dark photoperiod, while *G. mellonella* larvae were reared in constantly dark conditions. Honey drops containing 50% honey and 50% water were used as a food source for *B. hebetor*, while *G. mellonella* larvae were reared directly on honey combs and artificial diet.

The adults of the parasitoid were reared on the larvae of the greater wax moth *G. mellonella* as a host by using glass vials of (2 cm × 10 cm). Each vial contained 2 to 3, 5th instar larvae of the host and one female of the parasitoid; provided with cotton swabs/pads soaked in 50% honey and water as food source for *B. hebetor* adult. The females of the parasitoid started to parasitize the larvae of the host by first injecting a small quantity of paralyzing venom into mature (5th instar) larvae of the host before egg laying on them which induced partial or complete paralysis and then deposit between three to twenty eggs on the outside of the host body which later on develop into adult.

Experimental procedure

To evaluate the effect of cold temperature and storage duration on different life stages of *B. hebetor* 5th instar larvae of wax moth were separated from the laboratory culture and distributed in glass vials singly. A pair of *B. hebetor* was also released in each glass vial and allowed to parasitize. The females of the parasitoid started to parasitize the larvae of the host, after complete paralysis deposited three to twenty eggs on the outside of the host larvae body which later on develop into larvae, pupae and adult. After patriotization, cold storage experiments were carried out by placing the egg, larval, pupal and adult stage of *B. hebetor* at 4 ± 1°C for 5, 10, 20 and 40 days while 27± 1°C was used as control.

Transfer of host larvae to cold storage

Five replications were randomly allocated to the cold storage *i.e*., 4±1°C (having 50 eggs, larvae and pupae per 5thinstars host larvae (Total 250 specimens in each case) while adult was provided 50% honey solution in water. Each host stage was stored at 4±1°C temperature for four time durations (*i.e.*, 5, 10, 20 and 40 days). Cold conditions were maintained by placing the insects in growth chambers (1 set for cold storage (4±1°C) and other for control 27±1°C). After the purposed cold storage interval host larvae containing eggs, larvae and pupae of *B. hebetor* were removed from cold storage and placed at 27±1°C standard temperatures to check the effect of cold temperature and duration on the quality of parasitoids by measuring the following variables in replicates: number of survived to adult emergence, sex ratio and longevity of adult male and female wasp.

Data analyses

The data regarding analysis of variance were statistically analyzed by analytical software (2003) statistix 8.1 by using factorial design and the means were separated by LSD test at $p<0.05$.

RESULTS

Eggs of B. hebetor *and developmental stages*

Table I shows the effect of temperature and storage period on the mean number of emergence of larvae, pupae, adult, sex ratio and longevity of *B. hebetor*. Maximum mean number of larvae (ANOVA, df=3, F=267.86, 35.00±0.70, LSD test, P< 0.05, Table I) emerged at 4°C temperature after 5 days of storage followed by 30.0±2.00, 15.0±0.70 and 0.0±0.00 after 10, 20 and 40 days respectively, however their survival to adult stage was (ANOVA, df=3, F= 715.18, 30.0±1.58, 25.0±1.30 and 6.0±0.70, LSD test, P<0.05) respectively. Our findings showed that after 20 days cold storage only 15.00±0.70 eggs could hatch to larval stage which reduced to 0.0±0.00, P≤0.05 after 40 days storage period whereas in control (27±1°C) treatment 50.00±0.0 larvae were emerged followed by (48.0±0.70) pupae which survive to 47.0±0.70 adults after 10 and 20 days. After 40 days in control there was no adult emergence because all of them have completed their life cycles.

Female biased sex ratio was recorded after cold storage as 3.0±0.70 male/3.00±0.70 female emerged after 20 days treatment however in case of control male were more in number as compared to female, (ANOVA, df=3, F=2.20, 26.00±1.30 male and F=1.78, 21.00±1.00 female). The data regarding (Table I) longevity of both males and females was decreased with increase in storage time.

Developmental stages of the larvae of B. hebetor

Table II shows the effect of temperature and cold

Table I.- Effect of temperature and storage period on the eggs of *Bracon hebetor* and development stages.

Temperature (°C)	Storage period (Days)	No. of larva emerged	No. of pupa emerged	No. of adult emerged	Sex ratio		Longevity of adults	
					♀	♂	♀	♂
4±1°C	5	35.0±0.70b	30.0±0.70b	30.0±1.58b	14.0±1.00b	16.0±1.00b	22.0±1.30a	14.0±1.92a
	10	30.0±2.00c	30.0±1.14b	25.0±1.30c	12.0±0.70c	13.00±1.30c	16.00±1.00b	9.00±0.70b
	20	15.0±0.70d	10.0±1.14c	6.0±0.70d	3.00±0.70d	3.00±0.70d	10.00±1.3c	6.00±1.00c
	40	0.00±0.00e	0.00±0.00d	0.00±0.00e	0.00±0.00e	0.00±0.00d	0.00±0.00d	0.00±0.00d
27±1°C	5	50.00±0.00a	0.00±0.00d	0.00±0.00e	0.00±0.00e	0.00±0.00d	0.00±0.00d	0.00±0.00d
	10	0.00±0.00e	48.00±0.70a	0.00±0.00e	0.00±0.00e	0.00±0.00d	0.00±0.00d	0.00±0.00d
	20	0.00±0.00e	0.00±0.00d	47.0±0.70a	21.0±1.00a	26.0±1.30a	24.0±1.00a	15.0±1.00a
	40	0.00±0.00e	0.00±0.00d	0.00±0.00e	0.00±0.00e	0.00±0.00d	0.00±0.00d	0.00±0.00d

Table II.- Effect of temperature and storage period on the larvae of *Bracon hebetor* and development stages.

Temperature (°C)	Storage period (Days)	No. of pupa emerged	No. of adult emerged	Sex ratio		Longevity of adults	
				♀	♂	♀	♂
4±1°C	5	15.00±1.00b	10.00±1.41b	5.00±0.70b	6.00±1.00b	23.00±1.30a	13.00±1.41b
	10	4.00±1.00c	2.00±0.31c	1.00±0.31c	1.00±0.31c	15.00±1.3b	9.00±1.00c
	20	0.00±0.00d	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00d
	40	0.00±0.00d	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00d
27±1°C	5	50.00±0.00a	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00d
	10	0.00±0.00d	48.00±0.70a	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00d
	20	0.00±0.00d	46.00±1.41a	20.00±1.70a	26.00±1.30a	24.00±1.70a	15.00±1.41a
	40	0.00±0.00d	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.000d

storage period. The mean number of pupae formation, adult emergence, sex ratio and longevity of *B. hebetor*. Maximum mean number of pupae (ANOVA, df=3, F=778.36, 15.00±1.00, LSD test, P<0.05) was formed at 4°C temperature after 5 days of storage followed by (4.00±1.00and 0.00±0.00) after 10 and 20 days respectively however their survival to adult stage was (ANOVA, df=3, F=754.35, 10.00±1.41 and 2.00±0.31, LSD test, P<0.05, Table II) respectively. Our findings showed that after 10 days cold storage only (ANOVA, df=3, F=170.04, 2.00±0.31, LSD test, P<0.05) pupae was formed which reduced to (0.00±0.00, P<0.05) after 20 days storage period whereas in control (27±1°C) treatment 50.0±0.0 pupae were formed after five days which survived to 48.0±0.70 adult and completed their normal life cycle. After 40 days in control there was no adult emergence because all of them have completed their life cycles.

Female biased sex ratio was recorded after cold storage as 1.00±0.31 male/1.00±0.31 female emerged after 10 days treatment however in case of control male were more in number as compared to female (ANOVA, df=3, F= 149.73, 26.00±1.30 male and df=3, F=363.27 20.00±1.70 female). The data regarding (Table 1I) longevity of both male female was decreased with increase in storage time.

Developmental stages of pupae of B. hebetor

Table III shows the effect of temperature and storage period on the mean number of adult emergence, sex ratio and longevity of *B. hebetor*. Maximum mean number of adult (ANOVA, df=3, F=132.97, 40.0±1.14, LSD test, P<00.05) was emerged at 4°C temperature after 5 days of storage followed by 35.0±1.30, 30.0±1.00 and 20.0±1.41 after 10, 20 and 40 days, respectively. Our findings showed that after five days cold storage (ANOVA, df= 3, F=24.61, 40.0±1.14) adult was formed which reduced to 20.0±1.41, P<0.05 after 40 days storage period whereas in control (27±1°C) treatment 50.0±0.0 adults were formed after five days and completed their normal life cycles. After 40 days in control there was no adult because all of them complete their life cycle.

Female biased sex ratio was recorded after cold storage as (ANOVA, df=3, F=14.53, 11.00±1.41 female, whereas F= 19.30, 9.00±1.30 male emerged after 40 days treatment however in case of control male were more in number as compared to female *i.e*., 26.00±1.61 male and 23.00±1.30 female. The data regarding (Table III) longevity of both male female was decreased with increase in storage time.

Table III.- Effect of temperature and storage period on the pupae of *Bracon hebetor* and development stages.

Temperature (°C)	Storage period (Days)	No. of adult emerged	Sex ratio		Longevity of adults	
			♀	♂	♀	♂
4±1°C	5	40.00±1.14b	18.00±1.61bcd	22.00±1.61bc	23.00±1.61a	14.00±1.41a
	10	35.00±1.30c	17.00±1.30cd	18.00±1.92cd	16.00±1.61b	10.00±1.00b
	20	30.00±1.00d	15.00±1.30de	15.00±0.70d	11.00±1.61c	7.00±1.06c
	40	20.00±1.41e	11.00±1.41e	9.00±1.30e	9.00±1.30c	5.00±0.70c
27±1°C	5	50.00±0.00a	22.00±1.70ab	28.00±1.70a	24.00±1.30a	15.00±1.00a
	10	50.00±0.00a	23.00±1.30a	26.00±1.61b	26.00±0.70a	16.00±1.41a
	20	37.00±1.61c	21.00±2.23abc	25.00±0.70ab	25.00±2.00a	15.00±1.00a
	40	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00d	0.00±0.00d

Table IV.- Effect of temperature and storage period on the Adult of *Bracon hebetor* and development stages.

Temperature (°C)	Storage period (Days)	No. of adult emerged	Sex ratio		Longevity of adults	
			♀	♂	♀	♂
4±1°C	5	49.00±0.31a	25.00±0.70a	24.00±1.70a	24.00±1.92a	14.00±1.46a
	10	41.20±1.00b	23.00±1.41a	18.00±1.70b	16.00±1.30b	9.00±1.30b
	20	38.00±2.00c	22.00±1.61b	16.00±1.41c	10.00±1.41c	6.00±0.70c
	40	24.00±1.41d	14.00±1.14c	10.00±1.14d	8.00±1.30c	5.00±1.00c
27±1°C	5	50.00±0.00a	25.00±1.00a	25.00±1.70a	24.00±1.61a	15.00±1.00a
	10	47.00±1.02a	25.00±1.14a	22.00±1.00a	10.00±1.41c	5.00±1.00c
	20	24.00±1.30d	24.80±2.28a	0.00±0.00e	2.00±0.31d	0.00±0.00d
	40	0.00±0.00e	0.00±0.00d	0.00±0.00e	0.00±0.00d	0.00±0.00d

Adults of B. hebetor

Table IV shows the effect of temperature and storage period on the mean number of adult survived, sex ratio and longevity of *B. hebetor*. Maximum mean number of adult (ANOVA, df=3, F=80.11, 49.00±0.31, LSD test P<0.05) was survived at 4±1°C temperature after 5 days of storage followed by (41.20±1.46, 38.00±2.00 and 24.00±1.41) after 10, 20 and 40 days respectively. Our findings showed that after five days cold storage (ANOVA, df=3, F=4.93, 49.00±0.31, LSD test, P<0.05) adult was survived which reduced to (24.00±1.41, P<0.05) after 40 days cold storage period whereas in control (27±1°C) treatment all the 50.00±0.0 adults complete their normal life cycle. Male were died within 20 day storage while female died 25 days storage. After 40 days in control there was no adult emergence because all of them completed their life cycles.

Data recorded related to sex ratio after cold storage showed that after 40 days treatment more female survived as compared to male. Same results were observed in control that shows male have short life cycle as compared to female. The data regarding (Table IV) longevity of both male female was decreased with increase in storage time.

DISCUSSION

The present study was conducted to the check the effect of temperature and storage periods on different developmental stages of ectoparasitoid, *B. hebetor*. The results, showed that the survival of different stages (egg, larvae and pupae) of *B. hebetor* to adult stage decreased as the storage period increased however, the survival of pupae was more as compared to egg and larvae because they can survive (20.0±1.41) up to 40 days of cold storage while eggs and larvae can survive 15.0±0.70, 4.00±1.00 after 20 and 10 days storage respectively. Our results are in accordance with the studies of Payne (1934) and Franqui (1995) who reported that the eggs and larval stages are highly susceptible to cold temperatures. Van Lenteren and Tommasini (2002) also reported that pupal stage is more suitable for short term storage. There are experimental evidences showing that pupae are actually more cold tolerant than eggs, larvae (Jalali and singh, 1992; Nakama and Forester, 2001). In all stages (egg, larvae and pupae) data about the adult longevity, showed that the longevity of females and males decreased as the storage period increased at the same temperature. These results were supported by the studies of Farghaly and

Ragab (1993) who stored the pupae of *B. hebetor*, for 10, 15 and 30 days, respectively. Data regarding sex ratio shows that in all stage storage sex ratio was female biased with the increase in cold storage time duration. Our results are also in accordance with the findings of Yilmaz *et al*. (2007) who studied the effects of cold storage on the parasitization rate, longevity, emergence rate and sex ratio of cold stored adults of *Trichogramma evanescens* (Westwood, 1833) (Hymenoptera: Trichogrammatidae) he reported female biased sex ratios were observed for all storage periods in the parental and F1 generation. With the hymenopteran parasitoids, survival during and following cold storage generally favours the females regardless of the stage of development that was stored (Archer and Eikenbary, 1973; Hofsvang and Hågvar, 1977; Kovalenkov and Kozlova, 1981; Jackson, 1986; Zhu and Zhang, 1987; Zhang, 1992; Whitaker-Deerberg *et al*., 1994) also support our findings.

Our results (Table IV) showed that in comparison to all stages adult stage is the most suitable for storage at 4±1°C because their survival was more (*i.e.*, 24.00±1.41) compared to that of pupae (*i.e.*, 20.00±1.41) after 40 days storage period. Our findings are also supported by Uwais *et al*. (2006b) who reported that in cold storage treatment of *H. hebetor*, the mortality of pupae was much more than that of adults over the same duration of storage. Mousapour *et al*. (2014) reported that at 4°C, a significant reduction was caused in the efficacy of the stored pupae and is not recommended for cold storage.

CONCLUSION

It may be concluded that adult stage of *B. hebetor* is more cold tolerant than all other stages and considered to be more suitable for short term storage. On the other hand, number of individual survival decreases as the storage period increases along with rate of emergence.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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